

# SICKLE CELL REAGENT TEST KIT

For the qualitative determination of Hemoglobin S in blood

## INTENDED USE

For *in vitro* Diagnostic Use. For the qualitative detection of hemoglobin (Hb) S in blood using a phosphate solubility method.

## METHOD HISTORY

In 1910, Dr. Herrick of Chicago reported the "peculiar elongated and sickle shaped red blood corpuscle in the case of severe cell anemia" (1). This is the first known recognition of a case of sickle cell anemia and the origin of the name. Since that time, about 300 structural variants of the hemoglobin molecule have been described (2).

Although sickle cell disease is usually considered to be a particular affliction of Blacks, reaching a frequency of up to 59% in various regions of Africa, other ethnic populations may have forms of the disease. In some areas of the Middle East, Southern India, portions of South America and the Caribbean up to 50% of the population may be carriers. In the United States HbS is the most common hemoglobin variant; it is found in about 6 to 8% of Black Americans (3-5).

Itano (6) reported the poor solubility of deoxyhemoglobin in concentrated phosphate buffer. Several modifications of the original procedure have been reported (7-9). The Nova Century Scientific, Inc. procedure is a modified Nalbandian (7) procedure based upon phosphate solubility.

## METHOD PRINCIPLE

Erythrocytes are lysed by saponin and the released hemoglobin is reduced by sodium hydrosulfite in a phosphate buffer. Reduced HbS is characterized by its very low solubility and the formation of nematic liquid crystals (tactoids). The resulting tactoids of HbS or non-sickling hemoglobin (i.e. Harlem HbC) causes the solution to remain turbid. The presence of HbA under these same conditions results in a clear red solution. Electrophoretic confirmation is required for conclusive identification.

## REAGENTS

1. Sickle cell buffer of 0.97 M potassium phosphate monobasic, 1.33 M potassium phosphate dibasic with sodium azide as the preservative.
2. Sickle cell powder, a measured amount of sodium hydrosulfite and saponin.

**WARNING - Saponin - Strong Hemolytic Agent  
Sodium Azide - Laboratory Explosive Hazard**

## MATERIALS PROVIDED

The Sickle cell kit contains two bottles of sickle cell buffer, two vials of sickle cell powder, two dispensing closures and one reading card.

## MATERIALS NOT PROVIDED

Reagent and sample pipettes (20ul), test tubes (12 x 75mm). Negative and positive controls may be obtained from electrophoresis proven AA, AS, SS donors. Positive and negative controls may be purchased from Nova Century Scientific Inc. (Nova Century Scientific Inc. Sickle Cell Controls, 4 x 0.5 mL, Order No. NCS1).

## REAGENT STORAGE

The reagent set as received may be stored at 2-30°C. Do not freeze. Keep tightly capped to prevent evaporation of the buffer and to protect the powder from moisture. **After mixing, the sickle cell buffer must be stored at 2-8°C in the refrigerator.**

## CHEMICAL PRECAUTIONS

Sodium azide is a toxic and explosive compound and must be disposed of properly. Saponin is a powerful hemolytic agent. Wash hands after use and do not pipette by mouth.

## SPECIMEN COLLECTION AND HANDLING

Collect whole blood in a vial containing a suitable anticoagulant (heparin, EDTA, oxalate, or ACD, CPD, CPDA-1, and CP2D solution) or blood bank segments of whole blood or packed cells, mixed thoroughly, can be used as sample sources. Blood samples that have been kept for as long as one to six weeks at 1-8°C, are reported satisfactory (10).

Fresh whole blood from a skin puncture may also be used. If anemia or protein gammopathies are suspected, washing the RBC by centrifugal techniques may be useful in the preparation of the cells for assay.

## REAGENT PREPARATION

1. Add the entire contents of one sickle cell powder to the bottle of sickle cell buffer.
2. Mix thoroughly for about 2 minutes to assure complete reconstitution.
3. The reconstituted reagent must be stored at 1-8°C and kept tightly capped.

The working reagent is stable for 45 days under these conditions. Leaving reagent out at room temperature for greater than 24 hours will markedly reduce the working shelf life.

## PROCEDURE

1. Add 2.0 mL of working sickle cell reagent to premarked tubes labeled Unknown, Positive and Negative. Immediately return working sickle cell reagent buffer to the refrigerator.
2. Allow the tubes of reagent to warm to room temperature (approximately 5 minutes).
3. Add 20 uL of sample or controls or 10 µl of packed cells and mix by inversion.
4. Place in the sickle cell rack for 5-10 minutes.
5. Read the test by holding the tube approximately 3 cm in front of a line scale. If the solution is clear or a slight amount of turbidity is present and the lines are visible, the test is negative. If the solution is turbid and the lines are not visible, HbS or other non-sickling hemoglobin are present. In either case, electrophoretic confirmation is required for conclusive identification.

## PROCEDURE LIMITATIONS

Severe anemia will cause false negative results; therefore, if the hemoglobin concentration is 7g/dL or less, the sample test should be repeated using the procedure described below. Also, blood from patients with polycythemia, multiple myeloma, cryoglobulinemia and other dysglobulinemia may cause a false positive result (11).

If suspected conditions as described above are present, the red blood cells should be washed 2 times with blood bank saline and the test repeated with 10 uL of packed RBC sample.

Blood samples from infants from newborns until 6 months of age have up to 25% HbF present which may cause false negatives. Sickle cell solubility testing should not be used for this population of patients.

In all cases where hemoglobin abnormalities are indicated or suspected, electrophoretic confirmation is recommended.

## INDICATORS OF REAGENT DETERIORATION

1. Failure to obtain accurate results in the assay of control material.
2. Turbidity or crystals which will not readily dissolve upon mixing.
3. Prolonged room temperature exposure of the working reagent (24 hours or greater). Blood shows orange-red color.
4. Do not use sickle cell powder that has become damp.

Nova Century Scientific, Inc. cannot guarantee the stability of reagents which have been transferred from the original containers, improperly stored or contaminated during use.

## BIBLIOGRAPHY

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